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Pharmacology Review of 97-0201, Label Supplement to ReoPro JAN 22 1998

Introduction: $\alpha_v\beta_3$ receptor[1] for vitronectin possess a β chain in common with GPIIb/IIIa. Thus, antibodies to GPIIb/IIIa may be expected to cross-react with the vitronectin receptor and block the function of vitronectin. This review describes various studies which were conducted which to demonstrate the ability of c7E3 to block $\alpha_v\beta_3$.

1. Study: Technical Report: Functional implications of 7E3 binding to $\alpha_v\beta_3$. PRTR53v2.mtn. Centocor

The cross-reactivity of c7E3 Fab or m7E3 IgG with the $\alpha_v\beta_3$ was characterized by a number of different assays. The various assays tested for cross-reactivity as reflected by various endpoints from the level of a purified receptor to functional assays which reflected the biological activity of vitronectin. The findings of these studies demonstrates the ability of c7E3 Fab to react with the vitronectin receptor and block its function.

A. Binding to purified $\alpha_v\beta_3$ (vitronectin receptor). $\alpha_v\beta_3$ was purified from M21 melanoma cells and coated on the wells of polystyrene plates. ^{125}I-c7E3 Fab was then applied to the plate which were then washed and bound radioactivity was counted.

The affinity of c7E3 to purified $\alpha_v\beta_3$ was determined to be Ka = 1.05 X 10^8 M^{-1} (Kd = 0.48 μg/ml). LM609 IgG, an antibody to $\alpha_v\beta_3$, also bound to purified $\alpha_v\beta_3$, but not 10E5 IgG, an antibody to GPIIb/IIIa. m7E3 had a Kd = 0.41 μg/ml.

B. Binding Reactivity to Various Cells. Various primary human cell lines were grown to confluence in 96-well tissue culture plates. Cell lines included: umbilical vein endothelial cells (HUVEC), coronary artery endothelial cells, pulmonary artery smooth muscle cells, coronary artery smooth muscle cells, skeletal muscle cells, lung fibroblasts, epidermal keratinocytes, and bronchial epithelial cells. Binding to c7E3 Fab was determined using ^{125}I labeling procedures.

The following table lists the affinity constants for ^{125}I-c7E3 Fab in various cell types:

Human Cell Type	Affinity constants for ^{125}I-c7E3 Fab, Ka
Umbilical vein endothelial cells	0.88 X 10^8 M^{-1}

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[1]. $\alpha_v\beta_3$ mediates a number of cellular function including cell attachment and spreading. The receptor binds to a number of proteins associated with tissue injury: vitronectin, von Willebrand factor, fibrinogen, fibronectin, proteolyzed collagen, osteopontin, thrombospondin. $\alpha_v\beta_3$ is expressed on endothelial cells, fibroblasts, malignant melanoma cells, smooth muscle cells, NK cells, osteoclasts, and platelets. It is not expressed on normal skin or arteries; it is upregulated during angiogenesis, wound healing, and after balloon catheter injury.

Coronary artery endothelial cells	0.69 X 10^8 M^{-1}
Pulmonary artery smooth muscle cells	0.51 X 10^8 M^{-1}
Coronary artery smooth muscle cells	0.78 X 10^8 M^{-1}
Skeletal muscle cells	1.10 X 10^8 M^{-1}
Lung fibroblasts	1.34 X 10^8 M^{-1}
Keratinocytes	0
Bronchial epithelial cells	0

C. Cell Spreading Activity. Using glass chamber slides coated with various endogenous substrates were used to determine the antagonism of c7E3 Fab to cell spreading by HUVEC cells. Cells were incubated at room temperature with 10 μg/ml of an antibody and added to the chamber, incubated at 37° C, and photographically evaluated. c7E3 blocked adhesion to vitronectin (3+) and fibrinogen (6+), but not to fibronectin (1+) and gelatin (1+). Differences in the blockage for vitronectin vs fibrinogen may be due to vitronectin's effects being partial mediated by another vitronectin receptor, $\alpha_v\beta_5$. The results of the binding study are presented below (MT412 is a chimeric Fab fragment which binds CD4 and was used a negative control). The composition of attachment factor is not completely known, but is considered to be fibronectin rich. Cells were scored for spreading on a scale of '0' meaning no effect (no blockade) to ten '+' meaning all cells completely rounded (complete blockade).

Antibody	Vitronectin	Fibrinogen	Attachment Factor	Fibronectin	Gelatin
control	0	0	0	0	0
m10E5 IgG	0	++	0	0	0
cMT412 Fab	0	+	0	0	+
c7E3	+++	++++++	++++++	+	+
m7E3	ND	++++++	++++++	0	0
LM609 IgG	ND	++++++	++++++	0	+

D. Inhibition of Vitronectin Binding to M21 cells. Vitronectin was bound to the wells of a polystyrene plate to which ^{111}Indium labeled cells were added. Cells were washed and incubated with c7E3 Fab for 4 hrs at 37 °C. An anti-$\alpha_v\beta_3$ monoclonal antibody from ascites, clone P1F6

was used as a control. The incubated cells were placed on the vitronectin bound wells of the plate, washed, and bound radioactivity counted.

c7E3 and m7E3 IgG inhibited M21 adhesion to vitronectin coated plates with IC50 values of 0.10 μg/ml and 0.24 μg/ml. LM609 IgG also blocked binding (IC50 0.19 μg/ml) whereas 10E5 IgG did not. A maximal inhibition of 65% was observed due to the possible presence of an alternative binding site ($\alpha_v\beta_5$). Using a polyclonal antibody to $\alpha_v\beta_5$ allowed for complete inhibition in experiments which yield similar IC50 values for c7E3 (0.34 μg/ml) and m7E3 (0.37 μg/ml).

E. Antagonism of angiogenesis using a SCID mouse model. Human neonatal foreskin were sutured to the skin of 6 week old SCID mice. M21-L melanoma cells lacking $\alpha_v\beta_3$ were injected intradermally into the human skin. Two weeks later, a time before tumor growth is detectable, mice were given iv LM609 IgG (an $\alpha_v\beta_3$ blocking antibody), m7E3 IgG, or PBS. Mice continued to be treated and receive twice weekly injection for 3 weeks. At the study's conclusion, tumor volume and wet weight was determined.

A injection of LM609 IgG or m7E3 IgG resulted in a statistically significant reduction in tumor volume ($p=0.043$, $p=0.039$, respectively) as compared to controls. A similar effect was found on tumor weight which was not statistically significant, however.

Comment: c7E3 was not tested.

2. Study: Inhibition of Platelet-mediated Tissue Factor-induced Thrombin Generation by the Mouse/Human Chimeric 7E3 Antibody. J Clin Invest 98:863, 1996.

The effects of c7E3 and other antiplatelet agents were tested in a thrombin generation assay using tissue factor and gel filtered platelets. C7E3 Fab produced a dose dependent inhibition of thrombin generation at concentrations greater than or equal to 15 μg/ml. Inhibition reached a maximal effect of 48%. Antibody 10E5 (20 μg/ml) which blocks platelet GPIIb/IIIa decreased thrombin generation by about 23% 27%. Antibody LM609 (20 μg/ml) which blocks $\alpha_v\beta_3$ blocked thrombin generation by about 6%. The combination of c7E3 with LM609 was not more effective in blocking thrombin generation than c7E3 alone. The combination of 10E5 and LM609 inhibited 32% to 38% of the thrombin generated in comparison to control experiments. The lowest concentration of c7E3 tested was 5 μg/ml which yielded an inhibition of 18%. In contrast to the levels of c7E3 used in the studies on thrombin generation, plasma levels used in the therapeutic situation after a dose of 0.25 mg/kg are not likely to exceed 1.5 μg/ml as a peak concentration and sustained levels are less than 0.5 μg/ml.

Summary/Conclusion: c7E3 Fab is bound to purified $\alpha_v\beta_3$ with a Kd = 0.48 μg/ml whereas a value of 0.37 μg/ml was indicated by the sponsor for binding to HUVECs or 0.26 μg/ml for

platelets . Furthermore, c7E3 bound to a variety of human cell lines with a profile consistent the known distribution of $\alpha_v\beta_3$ with comparable affinities and reduced binding to vitronectin to M21 cells. Thus, c7E3 blocks $\alpha_v\beta_3$ as demonstrated by various receptor and cellular systems. Support of c7E3 blockade of angiogenesis was not specifically tested.

With regard to the proposed wording in the package insert:

A. Page 1, 1st paragraph. No changes are proposed to the sponsor's statement,

"Abciximab also binds with similar affinity to the vitronectin ($\alpha_v\beta_3$) receptors found on platelets and vessel wall endothelial and smooth muscle cells. The vitronectin receptor mediates pro-coagulant properties of platelets and proliferative properties of vascular endothelial cells and smooth muscle cells."

B. Page 1, 5th paragraph. Modifications to the following sponsor proposed text are indicated in italics and strikeout.

"Abciximab binds with similar affinity to the related integrin $\alpha_v\beta_3$, also known as the vitronetin receptor. The $\alpha_v\beta_3$ receptor is present on a wide variety of cell types including platelets and vessel wall enothelial and smooth muscle cells. *Using a model cell line derived from melanoma cells,* Abciximab ~~effectively~~ ~~blocks~~ *blocked* $\alpha_v\beta_3$ mediated effects including cell adhesion (IC50 = 0.34 µg/ml). ~~Because of its dual specificity, Abciximab~~ *At concentrations above the therapeutic range, Abciximab demonstrates in vitro a dual specificity which* more effectively blocks the burst of thrombin generation that follows platelet activation than ~~*experimental*~~ agents which inhibit GPIIb/IIIa alone.

C. Page 3, 2nd paragraph. No changes are proposed to,
"After discontinuation of Abciximab infusion, platelet function returns gradually to normal."

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